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The influence of critical surface tension and microrugosity on the adhesion of bacteria to polymer monofilaments

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Summary

The adhesion of *S. aureus* (NCIB 10788) and *E. coli* (NCIB 8196) to monofilaments of polypropylene (PP), polyethylene (PE), nylon (N) and polyvinylidine chloride (PVDC) was determined using [*methyl*-^{3}H]thymidine radiolabelled cells. Critical surface tensions were determined from Zisman plots and a microrugosity index developed using the waveform line from scanning electron micrographs of the substrate surface. *S. aureus* adhered in fewer numbers per cm^2 to PP than to PE, N or PVDC ($P < 0.05$) and adhesion of *E. coli* was less to PP and N than PE or PVDC ($P < 0.05$). *S. aureus* adhered to all monofilaments in greater numbers than *E. coli*. Critical surface tensions decreased in the order PVDC > N > PE > PP whereas the microrugosity indices of N and PE were greater than PP. PVDC could not be assigned a satisfactory microrugosity index since the monofilament possessed well-defined score marks on an otherwise 'smooth' surface. From critical surface tension measurements bacterial adhesion in vitro may have been expected to be the greatest to PP and least to PVDC: microrugosity of the substrate surface appears therefore to be a contributory factor in determining the extent of bacterial adhesion.

Introduction

Recent studies have highlighted several potential problems that exist as a result of the bacterial biofilms that form on the surfaces of medical implants. It has been reported that 'almost all postoperative wound infections are initiated along and in the vicinity of the suture lines' (Chu and Williams, 1984) and frequently demonstrated that the presence of suture material increases the likelihood of infection (Alexander et al., 1967; Blomstedt and Osteberg, 1978). Urinary catheters have been suggested to provide a vehicle for the growth of a multispecies bacterial biofilm from the exterior towards the bladder (Marrie and Costerton, 1983a). *Staphylococcus epidermidis* has also been suggested to become pathogenic due to an ability to adhere to, and grow along, Hickman catheters (Costerton et al., 1985). Similar colonisation of intrauterine contraceptive devices (IUDs) occurs in vivo and, post-removal, the surfaces have been found to be covered with a multispecies bacterial film several micrometers thick (Marrie and Costerton, 1983b). Indeed it has been speculated that endotoxin released from the millions of Gram-

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negative bacteria within this biofilm contributes to the inflammatory response of the endometrium of IUD users and hence to the contraceptive efficacy of these devices. Marrie and Costerton (1983b) also suggested that the presence of pathogenic species within such a biofilm may be a factor which leads to the increased risk of the development of pelvic inflammatory disease (PID) amongst IUD users (Mishell, 1985). PID is a microbial infection of the upper female genital tract which in some cases can cause infertility. Using an in vitro model, Wilkins et al. (1989) demonstrated the progression of various bacterial species along IUD marker tails and suggested that such threads may provide the route by which bacteria pass from the vagina to the normally sterile uterus. The extent of bacterial transmission appeared to be primarily determined by the motility of the organism, although adhesion to such surfaces was also of major importance. Scanning electron microscopy (SEM) was used to investigate this adhesion process (Wilkins, 1986). Organisms were seen to adhere reversibly at first and then to produce polymeric material to achieve stronger irreversible adhesion. The extent of bacterial adhesion was shown to be dependent upon factors which included the bacterial species, the composition of the surrounding milieu and the nature of the solid surface. Although SEM certainly provides a quick, easy and satisfactory method for assessing the numbers of bacteria adhering to a surface, the method can be associated with observer bias unless a rigid protocol is followed. The most satisfactory technique to assess bacterial numbers is an isotopic assay which, although expensive, is objective and reproducible (Mackowiak and Maling-Cason, 1984).

It is important to understand the process of bacterial adherence to solid substrata and the possible influencing factors in order to develop materials unsuitable for bacterial adherence. Our previous work has concentrated upon the problems associated with the presence of marker tails on IUDs (Wilkins, 1986; Wilkins et al., 1989). In this study, therefore, quantification of bacterial adherence to IUD marker tails has been studied using isotopic techniques and attempts made to relate the measurements to the critical surface tension (γ_c) of the substratum. Values of γ_c are dependent on both the atomic constitution and physical packing of atomic groups in the outermost surface layer of a solid. A method for semi-quantitatively assessing the microrugosity of the substrata was developed to investigate whether surface topography was a determinant of bacterial adhesion.

Materials and Methods

Bacteria

The bacteria studied were *S. aureus* NCIB 10788 and *Escherichia coli* NCIB 8196.

Media and chemicals

Mueller–Hinton broth (CM405) was obtained from Oxoid Ltd., Basingstoke, U.K. and prepared according to manufacturer's instructions.

Quarter-strength Ringer and phosphate-buffered saline (PBS) tablets were obtained from Oxoid Ltd. They were dissolved in distilled water according to manufacturer's instructions.

Cocktail T, sodium hydroxide (GPR), glycerol (Analar), and 1-chloronaphthalene (GPR) were obtained from BDH Chemicals Ltd., Poole, U.K.

Thiodiethane, diiodomethane and 1-bromonaphthalene were obtained from Aldrich Chemical Company, Gillingham, Dorset, U.K.

1-Methylnaphthalene (pure) was obtained from Koch Light Laboratories, Colnbrook, U.K.

[*methyl*-^{3}H]Thymidine, 6.7 Ci/mmol (batch nos. 2161-204, 2161-293) was obtained from New England Nuclear, Southampton, U.K.

Polymer threads

Polyvinylidine chloride (PVDC) was obtained from Nymofil Ltd., Poulton-le-Fylde, U.K.

Nylon monofilament from the Progestasert IUD was obtained from Alza Pharmaceuticals, Palo Alto, CA, U.S.A.

Polyethylene monofilament from the Lippes loop IUD was obtained from Ortho-Cilag Pharmaceuticals Ltd., High Wycombe, U.K.

Polypropylene monofilament from the Multiload CU250 IUD was obtained from Organon Laboratories Ltd., Morden, U.K.

All threads were treated by placing under a bactericidal ultraviolet unit (Hanovia Lamps, Slough, U.K.) for 1 h.

Assessment of bacterial adhesion

Cultures of radiolabelled *S. aureus* and *E. coli* were obtained by inoculating 25 ml Mueller–Hinton broth containing 0.25 ml of the radiolabel, [*methyl*-^{3}H]thymidine (6.7 Ci/mmol, 1 mCi/ml), with a single bacterial colony and then incubating without shaking overnight at 37 °C. Organisms were collected by centrifugation (3000 *g* for 20 min) and then washed three times in $\frac{1}{4}$ strength Ringer. Bacteria were resuspended in $\frac{1}{4}$ strength Ringer and their concentration adjusted photometrically to $0.9–1.8 \times 10^8$ cfu/ml by reference to previously constructed calibration curves. Three 0.2 ml aliquots of the bacterial suspension were taken and 19.8 ml cocktail T added to each for determination of counts per minute (cpm) in a liquid scintillation counter (model LS31 33P; Beckman Instruments Inc., Fullerton, CA). Aliquots (10 ml) of the bacterial suspension were added to each of 10 centrifuge tubes containing 5 monofilaments (8 cm length each), i.e., 50 threads in total for each experiment. These were incubated for 2 h in a shaking water bath (114 throws/min) at 37 °C. After incubation, the monofilaments were removed and washed 3 times in $\frac{1}{4}$ strength Ringer. Fluid from the final wash was shown not to contain cpm above background levels. The monofilaments were then incubated in a centrifuge tube containing 10 ml of 0.1 N NaOH for 1 h in a shaking water bath (114 throws/min) at 37 °C to remove adherent bacteria. The monofilaments were removed and three 3-ml aliquots of the final NaOH solution were taken and 17 ml cocktail T added to each for radiolabel determination using liquid scintillation counting. Glass scintillation vials (20 ml) were used throughout. Threads investigated were polypropylene, polyethylene, nylon and PVDC.

All cpm values were converted into decays per minute (dpm) through the use of appropriate quench correction curves. Typical percentage efficiencies were in the range 30–40% for the $\frac{1}{4}$ strength Ringer aliquots and 25–35% for the 0.1 N NaOH aliquots. The dpm values for the known concentration bacterial suspensions were then converted into dpm per bacterium (typical results giving 1 bacterium $= 1.5 \times 10^{-3}$ dpm) and from these values it was possible to calculate the number of bacteria adhering per cm^2 thread surface.

Critical surface tension determinations

The critical surface tensions of IUD threads and PVDC monofilament were calculated by determining the contact angles of a series of liquids placed on the polymer surface. A length of thread was placed on a platform sited between the condenser and lens of a projector. Using a microsyringe, a small drop of an organic liquid was placed onto the thread surface and its magnified image was projected onto a screen situated approximately 1.5 m away. The contact angle for the liquid on the polymer was measured from the image, the value of the angle being dependent upon the properties of both the liquid and the solid. Contact angles for a series of organic liquids (glycerol, thiodiethane, diiodomethane, 1-bromonaphthalene, 1-chloronaphthalene, 1-methylnaphthalene) on each polymer monofilament were measured. The lamp in the projector was switched off after each angle determination to prevent undue heating of the sample. All measurements were made at room temperature (approx. 16 °C).

Liquid surface tension determinations

Liquid surface tensions were determined at room temperature using a Du Noüy tensiometer (Cambridge Scientific Instruments Ltd., Cambridge). The platinum ring and glass Petri dish used to contain the liquids were cleaned using chromic acid and rinsed with double distilled water after each set of liquid measurements.

Assessment of microrugosity

Thread sections (1 cm length) were attached to metal stubs, sputter gold-coated (D.C. Sputter coater; EM Scope, Ashford, U.K.) and observed under a Scanning Electron Microscope (model ISI-100A; International Scientific Instruments, Manchester). Photographs were taken of the whole surface and also of the waveform monitor lines of small selected areas. Since the waveform monitor line represents a single scan line of the sample

surface it was employed as a measure of surface topography. Five areas ($\times 200$ magnification) were chosen randomly along the length of the threads, one quarter of the way in from the thread edge so that the angle of incidence of the electron beam to the sample surface was approx. 60°, in order to enhance the secondary electron transmission. Photographic transparencies of the waveform monitor line were subsequently projected onto a 3 m wide screen and the length measured by planimetry. An index of microrugosity was obtained for each thread by using the formula:

$$\text{Index of microrugosity} = \frac{\text{length of waveform line}}{\text{length of waveform line for perfectly smooth surface}}$$

Results

Bacterial adhesion

The extent of adhesion of bacteria to the various thread surfaces is shown in Table 1. *S. aureus* was found to adhere in greatest numbers to PVDC monofilament with approx. 90, 80 and 20% of this number adherent to nylon, polyethylene and polypropylene, respectively. These data and all subsequent data obtained from the isotopic method were analysed using the two-tailed Mann-Whitney *U*-test for unpaired non-parametric data (Siegal, 1956). The adhesion of *S. aureus* to PVDC, nylon and polyethylene threads was significantly higher than the adhesion to polypropylene thread ($P < 0.05$). Significantly fewer *E. coli* cells were found to adhere to each thread type as compared to *S. aureus* ($P < 0.05$). The highest number of *E. coli* cells attached to PVDC with approx. 85, 20 and 20% of this number adhering to polyethylene, polypropylene and nylon, respectively. The adhesion of *E. coli* to polyethylene and PVDC was significantly higher than that to nylon and polypropylene threads ($P < 0.05$).

TABLE 1

Adhesion of S aureus and E. coli to polymer monofilaments using [methyl-^{3}H]thymidine radiolabelled cells

Thread type	Mean number of adherent cells per cm^2 thread surface ± S.E. ($n = 4$)	
	S. aureus	*E. coli*
PVDC	$3.27 \pm 0.35 \times 10^6$	$3.19 \pm 0.32 \times 10^5$
Nylon	$2.86 \pm 0.15 \times 10^6$	$6.67 \pm 0.81 \times 10^4$
Polyethylene	$2.57 \pm 0.25 \times 10^6$	$2.71 \pm 0.32 \times 10^5$
Polypropylene	$6.71 \pm 0.71 \times 10^5$	$7.60 \pm 0.98 \times 10^4$

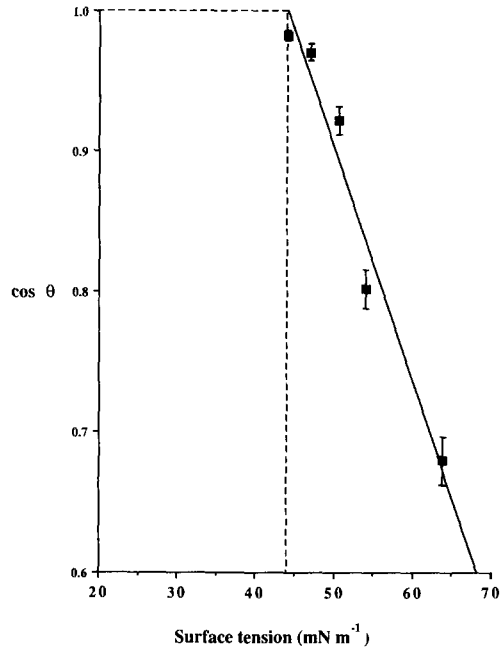

Fig. 1. Zisman plot for nylon monofilament. The value of liquid surface tension (mN m^{-1}) corresponding to cos $\theta = 1$ is the critical surface tension. Similar plots for polyethylene, polypropylene and PVDC were obtained but are not shown.

Critical surface tension determinations

To minimise experimental error, extremely small drops of organic liquids were utilised for

TABLE 2

Critical surface tensions of polymer monofilaments calculated from Zisman plots

Monofilament type	γ_c (mN m^{-1})
PVDC	48.38
Nylon	44.30
Polyethylene	41.95
Polypropylene	39.00

TABLE 3

Indices of microrugosity for polymer monofilaments

The values for nylon and polyethylene are not significantly different. The remainder are significantly different from each other ($P < 0.05$).

Monofilament type	Mean index ($n = 5$) ($\pm$S.D.)
Nylon	1.478 ± 0.083
Polyethylene	1.426 ± 0.059
PVDC (scored region)	1.237 ± 0.054
Polypropylene	1.124 ± 0.023
PVDC (smooth region)	1.072 ± 0.029

contact angle measurements in order to eliminate gravitational distortion of shape. θ is then independent of the liquid drop volume and temperature variations do not influence results unduly. Values obtained from the measurements of the contact angles for the various organic liquids on thread surfaces were recorded. Zisman plots were made of cos θ values against the liquid surface tension for each thread (Fig. 1). An example of such a plot, for nylon, is shown in Fig. 1. Extrapolation of the line to cos $\theta = 1$ gives a value of the critical surface tension of the monofilament and these values are shown in Table 2.

Microrugosity

Nylon and polyethylene monofilaments had greater microrugosity indices than the polypropylene thread, the rank order of increasing roughness being polypropylene < polyethylene < nylon. PVDC monofilament was more difficult to classify (Table 3) since it exhibited extensive smooth areas but with distinct score marks.

Discussion

Many of the values of critical surface tension (Table 2) for the polymer monofilaments differed from those reported previously for pure materials. Typical values are polypropylene, 29 mN/m; polyethylene, 31 mN/m; PVDC, 40 mN/m; nylon 42.5–46 mN/m (Baier et al., 1968; Hench and Ethridge, 1982). However, it should be appreciated that the monofilaments employed in this study were of commercial grade and therefore contained a number of different chemicals introduced at the time of manufacture. These include dye materials, to render the monofilaments easily visible, and plasticisers, which act as lubricants between molecular chains thereby rendering a high flexibility to the thread. Such materials will alter the characteristics of the polymer and affect the values of the critical surface tension.

In general, it is considered that critical surface tensions obtained from measurements with a variety of liquids are very useful parameters which are characteristic of the solid only (Baier, 1970). At the very least, they provide an empirical ranking of materials according to their relative surface energies (Baier, 1970; Tadros, 1980). The extent of bacterial adhesion to surfaces has, in many previous studies, been related to the substratum surface free energy (or some associated parameter).

The extent of bacterial adhesion to solid surfaces in vitro is often found to be highest on low-wettability surfaces (i.e. a low critical surface tension), decreasing with increasing wettability (Fletcher and Loeb, 1979). If critical surface tension was the major influencing factor then the extent of adhesion would be expected to decrease in the order polypropylene > polyethylene > nylon > PVDC. In fact the adhesion of *S. aureus* to monofilaments was found to increase with increasing wettability; the lowest number of cells adhering to polypropylene with increased numbers to polyethylene, nylon and PVDC (Table 1). The adhesion of *E. coli* was also found generally to increase with increasing surface wettability, with the exception of adhesion to nylon.

All estimates of surface free energy and critical surface tension dependent upon measurements of contact angles are at best an average property of the surface since microhomogeneities are not taken into account. Several workers have stressed that too little attention has been given to the effects of surface roughness (Baier et al., 1968; Busscher et al., 1984). Scanning electron microscopy was therefore used to investigate the microrugosity of IUD threads and PVDC monofilaments. Nylon and polyethylene threads were classed as having extremely rough grooved surfaces whereas polypropylene thread was much smoother. PVDC

monofilament had unusual surface characteristics, with extensive smooth areas and distinct score marks; presumably caused by the extrusion process during manufacture. It is proposed that microrugosity is one of the determinants of bacterial adhesion. For example, the adhesion of *S. aureus* to the relatively smooth surface of the polypropylene monofilament was significantly less than to the other monofilaments. This finding is confirmed by a previous study where scanning electron micrographs showed bacteria adhering in greater numbers to the rougher as opposed to the smoother areas of thread surfaces (Wilkins, 1986). The interrelationship between critical surface tension and microrugosity as factors affecting the extent of bacterial adhesion is undoubtedly complex and dependent upon the type of adsorbing micro-organism (Table 1) and composition of the surrounding bathing medium (Wilkins, 1986). In addition although useful information can be gained from in vitro adhesion studies, it is acknowledged that problems arise when trying to extrapolate such studies to the in vivo situation. The surfaces that bacteria encounter in vivo will undoubtedly be different from those investigated in vitro since all surfaces in natural environments are coated with a layer of organic macromolecules. For example, bubble contact angles at surfaces exposed to various bathing fluids were found to vary with the composition and concentration of the macromolecular solutions, as well as with the substratum characteristics (Fletcher and Marshall, 1982). Also, the wetting properties, as judged in the dry state by the use of contact angles, of unused Lippes loops were markedly different from those which had been used (Baier and Lippes, 1975).

Finally, adhesion is not the only factor which must be considered in the transmission of bacteria along polymeric threads, *E. coli* for example was shown to progress through gels using the monofilaments as substrata at the following decreasing rates: polyethylene > polypropylene > nylon and PVDC whereas the similar ranking for *S. aureus* was nylon > polyethylene > polypropylene > PVDC (Wilkins et al., 1989). The flagellated *E. coli* progressed faster than the non-motile *S. aureus*. More basic research on the physiochemistry of substrates is thus necessary to define further the factors of importance in determining the degree and extent of both bacterial adhesion to and transmission along monofilaments of medical importance.

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